

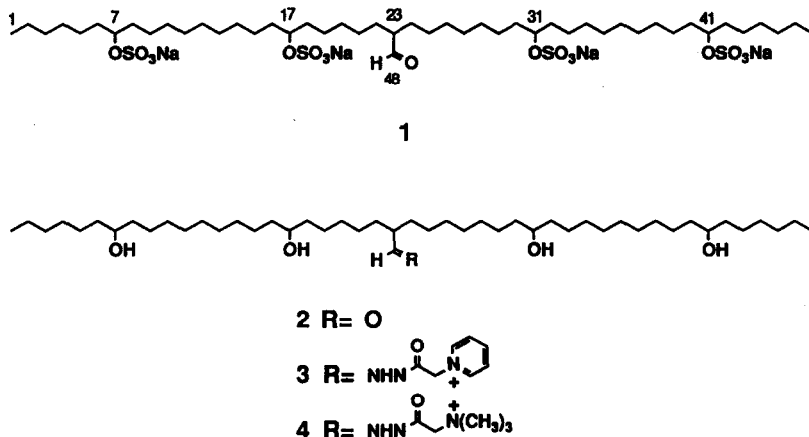
Toxadocial A: A Novel Thrombin Inhibitor from the Marine Sponge *Toxadocia cylindrica*¹

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Abstract. A unique thrombin inhibitor, named toxadocial A (1) has been isolated from the marine sponge *Toxadocia cylindrica*, and its structure was determined by spectroscopic and chemical methods to be a per-sulfated 7, 17, 31, 41-tetrahydroxyheptatetracontane-23-carbaldehyde.

Sulfated metabolites have rarely been encountered in marine sponges; examples are sterols,² prenylated hydroquinones,³ and halenaquinol sulfates.⁴ These compounds exert a variety of biological activities including antimicrobial, H, K-ATPase and PLA₂ inhibitory. In our continuous studies of bioactive metabolites from Japanese marine invertebrates, we collected the marine sponge *Toxadocia cylindrica*, whose hydrophilic extract showed potent inhibitory activity against thrombin.⁵ Bioassay-guided isolation afforded a unique sulfated C₄₇ aldehyde, toxadocial A (1). This paper deals with isolation and structure elucidation of this new type of compound.

The MeOH extract of the frozen sponge (1.9 kg, wet weight), collected off Hachijo-jima Island (-5~-20 m), was defatted with Et₂O, followed by extraction with *n*-BuOH. The MeOH soluble portion of the *n*-BuOH extract was gel-filtered on Sephadex LH-20 with MeOH. After abortive attempts to purify the active fractions by HPLC on ODS and polystyrene resins with various solvent systems, we finally succeeded in isolating pure toxadocial A as a colorless solid (55.2 mg, 2.9×10⁻³ % based on wet weight) along with its congeners⁶ by using ODS and aqueous MeCN containing NaClO₄ as a solvent.

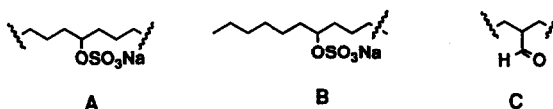


Toxadocial A (1)⁷ contained sulfate groups which was inferred from an intense IR band at 1250 cm⁻¹, as well as by a positive sodium rhodizonate test.⁸ This was supported by a deshielded methine signal (δ H 4.31

quint, δ_C 81.0 d) equivalent to four methines in the NMR spectra. In addition to four sulfated methines, the 1H and ^{13}C NMR spectra demonstrated two terminal methyls (δ_H 0.89 t, δ_C 14.4 q), a methine (δ_H 2.22 m, δ_C 53.2 d), an aldehyde (δ_H 9.51 d, δ_C 207.6 d), and alkyl chains. The aldehyde group was equilibrated with an hemiacetal (δ_H 4.38, δ_C 101.4) in CD_3OD .

Determination of the molecular formula for **1** was a key step in the structure determination. The negative ion FAB mass spectrum using *m*-nitrobenzyl alcohol or glycerol as a matrix exhibited ion peaks at m/z 1137, 1035 and 933; the latter two ions were formed by sequential loss of $NaSO_3$.^{2c} Therefore, the ion at m/z 1137 was an $(M-Na)^-$ ion, which together with NMR data led to a molecular formula of $C_{48}H_{92}O_{17}S_4Na_4$ (molecular weight, 1160). When diethanolamine was used as a matrix, ion peaks were shifted to m/z 1224, 1122, and 1020, respectively, which were 87 mu higher than those observed with *m*-nitrobenzyl alcohol or glycerol. This can be rationalized by the reaction of diethanolamine with the aldehyde group of the molecule to generate a Schiff's base, which rearranged to an enamine during ionization in the mass spectrometer.

Interpretation of NMR data including COSY, HOHAHA,⁹ and HMQC spectra¹⁰ was hampered due to severely overlapping signals, e.g., four oxymethine signals. Fortunately, an HMQC-HOHAHA spectrum,¹¹ which gave signals good enough to deduce correlations, led to units A, B, and C; A and B were duplicated. The oxymethine protons correlated with methylene carbons at δ 35.3, 30.8, and 26.0 in the HMQC-HOHAHA spectrum, thus placing all oxymethines in the middle of alkyl chains of similar environment to construct unit A. Besides a large methylene envelope at δ 30.8, the ^{13}C NMR spectrum exhibited two-carbon methylene signals at δ 35.3, 33.0, 30.5, 26.1, and 23.7, all of which correlated with terminal methyl protons in the HMQC-HOHAHA spectrum, thereby defining unit B. An aldehyde was attached to a methine carbon flanked by methylene carbons at δ 29.9 and 28.1 (unit C). Because of repetitive units in the molecule, elucidation of the gross structure was impossible by NMR experiments. Therefore, we undertook some chemical transformations.



Toxadocial A was hydrolyzed with 1 N HCl (100 °C, 30 min) to afford tetraol **2** as a colorless amorphous solid.¹² A molecular formula of $C_{48}H_{96}O_5$ was established by HRFABMS [m/z 753.7359 (M+H)⁺], which confirmed the molecular formula of the parent compound. Again NMR data led to structural units A-C, which were also supported by a FAB-MS/MS experiment.¹³ However, no further information was obtained. A tetraacetate provided no useful information. We then turned our attention to the aldehyde group with which a Schiff's base could be generated.¹⁴ Girard's reagents T and P¹⁵ were employed to facilitate analysis of fragment ions; ions arising from cleavage at the same site would differ by 20 mu for the two derivatives. The FAB-MS/MS spectrum of the Girard's reagent P derivative **3** showed intense ions at m/z 800, 770, 664, 614, 558, and 530 (Fig. 1), while the Girard's reagent T derivative **4** led to corresponding ions 20 mu smaller, thus allowing assignment of the positions of four hydroxyl groups and an aldehyde. To confirm this assignment we ran FABMS/MS/MS experiments with daughter ions of **3** at m/z 558 and 530; ion at m/z 558 yielded fragment ions at m/z 472 (C22-C41) and 442 (C22-C40), whereas ion at m/z 530 led to ions at m/z 444 (C8-C24) and 414. Thus, structures proposed for **1** and **2** were correct.

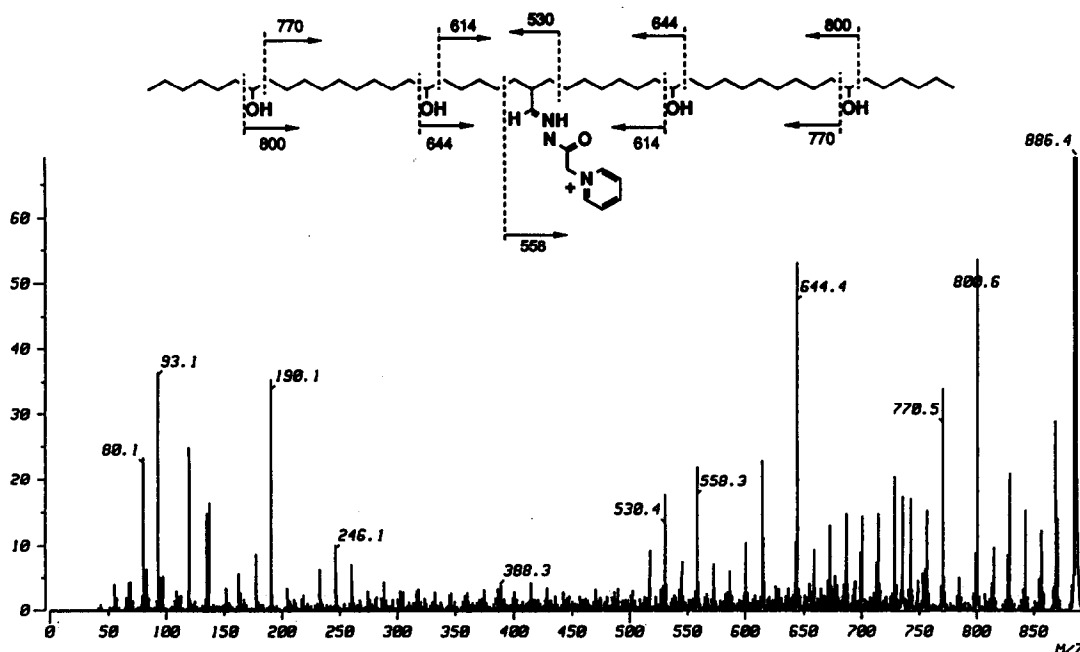


Fig.1. FABMS/MS of the Girard's P derivative.

To our knowledge toxadocial A is the first example of this unique class of compounds, though some remotely related compounds have been isolated from terrestrial microbes and echinoderms; izumenolide, a sulfated lactone from *Micromonospora chalcone* subsp. *izumensis*¹⁶ and a bis-sulfate from the starfish *Asterias forbesi*.¹⁷ Toxadocial A inhibited thrombin with an IC₅₀ of 6.5 μg/mL.

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References and Notes

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 6. The structures of the congeners will be reported elsewhere.
 7. 1: $[\alpha]_D -2.2^\circ$ (c 1.0, MeOH); FABMS (neg, *m*-nitrobenzyl alcohol) m/z 1137 (M-Na)⁻, 1035, 933; IR (film) 3450, 2940, 2860, 1720, 1250, 1070, 940 cm⁻¹; ¹H NMR (CD₃OD) δ 9.51 (d, *J*=3.0 Hz, H-48), 4.31 (quint, *J*=4.9 Hz, H-7, H-17, H-31, H-41), 2.22 (m, H-23), ~1.65 (m, H-6, H-8, H-16, H-18, H-30, H-32, H-40, H-42), 1.63~1.46 (m), ~1.39 (m, H-5, H-9, H-15, H-19, H-29, H-33, H-39, H-43), ~1.3 (m, H-2~4, H-10~14, H-20, H-24~28, H-34~38, H-44~46), 0.89 (t, *J*=6.7 Hz, H-1, H-47); ¹³C NMR (CD₃OD) 207.6 (d, C-48), 81.0 (d, C-7, C-17, C-31, C-41), 53.2 (d, C-23), 35.3 (t, C-6, C-8, C-16, C-18, C-30, C-32, C-40, C-42), 33.0 (t, C-3, C-45), 30.8 (t, C-10~14, C-20, 21, C-25~28, C-34~38), 30.5 (t, C-4, C-44), 29.9, 28.1 (t, C-22, C-24), 26.0 (t, C-5, C-9, C-15, C-19, C-29, C-33, C-39, C-43), 23.7 (t, C-2, C-46), 14.4 (q, C-1, C-47).
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 12. a) 2: $[\alpha]_D -3.7^\circ$ (c 0.175, CHCl₃); FABMS (pos, *m*-nitrobenzyl alcohol) m/z 753 (M+H)⁺; HRFABMS m/z 753.7359 [(M+H)⁺; C₄₈H₉₇O₅, Δ 2.3 mmu]; ¹H NMR (CD₃OD) 9.53 (d, *J*=3.0 Hz, H-48), 3.57 (4H, m, H-7, H-17, H-31, H-41), 2.22 (m, H-23), ~1.41 (m, H-6, H-8, H-16, H-18, H-30, H-32, H-40, H-42), ~1.3 (m, H-2~5, H-9~15, H-18~22, H-24~29, H-33~39, H-43~46), δ 0.89 (6H, t, *J*=6.7 Hz, H-1, H-47).
 b) HPLC analysis of the water soluble portion of the hydrolyzate revealed the presence of 4 sulfate groups in 1. (Murata, M.; Kumagai, M.; Lee, J. S.; Yasumoto, T. *Tetrahedron Lett.* **1987**, *28*, 5869-5872.)
 13. Negative FABMS/MS experiment of the (M-H)⁻ ion of 2 at m/z 751 gave rise to daughter ions at m/z 733, 665, 635, 509, 491, and 479.
 14. Due to the positive charge of the base, fragment ions incorporating a Schiff's base could be readily observed.
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